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Development and evaluation of an artificial membrane for determination of drug availability

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Abstract

Various artificial membranes (e.g. PAMPA) and cellular-based membranes (e.g. Caco-2) are used for screening during early stages of drug discovery. However, these methods are not well suited for evaluation of pharmaceutical formulations and the effects of various excipients on drug availability. When drug molecules permeate biological membranes they encounter two types of permeation resistance, a membrane resistance in the lipophilic membrane and diffusion resistance in the unstirred water layers adjacent to both surfaces of the lipophilic membrane. We have developed an artificial membrane that is cheap and simple to prepare. The unstirred water layer consists of a hydrated semi-permeable cellophane membrane with a molecular weight cutoff (MWCO) of 12,000–14,000 Da and a lipophilic membrane of pure *n*-octanol in a nitrocellulose matrix. In the diffusion cell the hydrated cellophane membrane (thickness 210–230 μm) is on the donor side and the lipophilic octanol membrane (thickness about 120 μm) on the receptor side. Permeation of ionizable lipophilic drug molecules was diffusion-controlled when the drug was unionized but lipophilic membrane controlled when the drug was ionized. Drug permeation patterns from cyclodextrin containing formulations through the membrane were similar to those previously observed for biological membranes such as hairless mouse skin and the eye cornea.
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Keywords: Octanol membrane; Permeation; Unstirred water layer; Formulation; Cyclodextrin; Availability

1. Introduction

Lipophilicity and permeability are two of the key physicochemical properties of new compounds determined during early stages of drug discovery. Other physicochemical properties determined during high-throughput screening include integrity, pK_a and aqueous solubility (Kerns and Di, 2004). Traditionally lipophilicity is expressed as the logarithm of the partition coefficient of a compound between *n*-octanol and water or between *n*-octanol and an aqueous buffer solution ($\log K_{o/w}$), and the coefficient relates to the compounds preference for interactions with other organic molecules versus hydrogen bonding and dipole interactions with water. Frequently, small lipophilic molecules (MW < approximately 600 Da) have low aqueous solubility but relatively good permeability through lipophilic membranes. However, for optimum bioavailability the drug candidate must possess sufficient solubility in the aqueous membrane exterior and, thus, for homologous series of compounds maximum absorption through a biological membrane is obtained at some optimum $\log K_{o/w}$. The most common pathway for drug absorption is transcellular passive diffusion. In general, parallel artificial membrane permeability assay (PAMPA) is used for early screening of passive diffusion but the more complex Caco-2 and other cell-based assays are used for later screening and evaluation of transporter-mediated permeability of selected compounds (Kerns and Di, 2004). In PAMPA the permeability is determined through a microfilter that has been impregnated with a solution of phospholipids in dodecane (Kansy et al., 1998; Ruell et al., 2003). Somewhat similar artificial membrane assay method has recently been described where the dodecane solution is replaced by an octanol solution of phospholipids and cholesterol (Corti et al., 2006a, 2006b) or pure octanol (Faller et al., 2005). It has been shown that PAMPA is as good indicator for oral drug absorption as Caco-2 (Zhu et al., 2002; Bermejo et al., 2004). The structural characteristics of *n*-octanol (i.e. lipophilic carbon chains and hydrophilic hydroxy groups) and its ability to form hydrogen bonds and take up water molecules, as well as its solubility parameter, renders *n*-octanol properties that are

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very close to those of phospholipid-based biological membranes (Panchagnula and Thomas, 2000) and, thus, *n*-octanol is used in high-throughput screening of lipophilicity. The aim of this study was to develop and evaluate *n*-octanol membrane for evaluation of drug permeability and drug availability in pharmaceutical formulations. The membrane should have sufficient strength to be used in normal size Franz diffusion cells. The lipophilic membrane should be protected from various excipients in the donor phase by a thin hydrophilic layer and, thus, mimicking various biological membranes such as the eye cornea and buccal mucosal membrane.

In PAMPA a microfilter is impregnated with a lipophilic solvent and phospholipids. Alternatively polymers can be used to convert solvents to non-aqueous gels or membrane matrixes. For example, Neubert and co-workers have used pyroxylin, which is partly nitrated cellulose, to form dodecanol membrane matrix for drug permeability studies (Neubert and Fürst, 1989; Chrzanowska et al., 2003; Mrestani et al., 2003, 2004). The membrane is prepared by mixing dodecanol with collodion, which is a solution of nitrocellulose (pyroxylin) in a mixture of ether and ethanol, and after solvent evaporation the membrane formed consists of equal amounts (w/w) of dodecanol and nitrocellulose. In this present study very similar methods were applied to prepare *n*-octanol membranes.

Adjacent to membrane surfaces is an unstirred or stagnant water layer that acts as diffusion barrier for rapidly permeating drugs. The thickness of this diffusion barrier and its significance in the overall barrier function of a given membrane depends on the physicochemical properties of both the membrane and the penetrating drug molecule. The thickness of the unstirred water layer has been estimated to be 1462 μm in PAMPA (Avdeef et al., 2004), 1544 μm in Caco-2 (Karlsson and Artursson, 1991) and 30–100 μm in the gastrointestinal tract (Lennernäs, 1998). The total drug permeation resistance is the sum of resistance within the unstirred water layer and the lipophilic membrane, and their relative importance depends on the physicochemical properties of both the drug and the membrane (Flynn et al., 1972; Florence and Attwood, 1998; Masson et al., 1999; Loftsson and Masson, 2001). In PAMPA, it has been shown that for lipophilic molecules, the unstirred water layer is the rate-limiting barrier to transport (Bermejo et al., 2004; Nielsen and Avdeef, 2004). However, the apparent thickness of the unstirred water layer in PAMPA varies for different molecules (Avdeef et al., 2004). Furthermore, according to the Stokes–Einstein equation the value of the diffusion coefficient decreases (i.e. the resistance increases) with increasing viscosity of the unstirred water layer and, thus, the effective resistance of the relatively viscous aqueous mucin layer, for example in the gastrointestinal tract, nasal mucosa or on the eye cornea, can be significant and much greater than expected based on the thickness of the unstirred layer. In this study it was attempted to standardize the thickness and the viscosity of the unstirred diffusion layer by fusing together a hydrated semi-permeable cellophane membrane and the lipophilic octanol membrane. This type of artificial membrane will, for example, be better suited than the currently used artificial membranes for investigation of how various vehicle excipients affect drug availability in pharmaceutical formulations.

2. Materials and methods

2.1. Materials

2-Hydroxypropyl-β-cyclodextrin of molar substitution 0.64 (HPβCD) was purchased from Roquette (Lestrem, France), hydrocortisone from ICN Biomedicals Inc. (OH, USA), lidocaine hydrochloride from Bufa (Uitgeest, Netherlands), naproxen from Lyfjaverslun Islands (Reykjavik, Iceland), 17β-estradiol from Pharmatec Inc. (Alachua, USA), sodium nitrate and ethanol from Merck (Darmstadt, Germany), salicylic acid from Norsk Medisinaldeport (Oslo, Norway), *n*-octanol (99%) and triclosan were purchased from Sigma (St. Louis, USA), and *n*-dodecanol, collodion (containing 4–8% nitrocellulose) and diethyl ether from Fluka (Hamburg, Germany). The cellophane membranes used were Spectra/Pore membrane from Spectrum Laboratories Inc. (Rancho Dominguez, USA) with molecular weight cutoff (MWCO) 3500, 6000–8000 and 12,000–14,000 Da. All other reagents were of analytical or special reagent grade.

2.2. Membranes

Collodion, nitrocellulose in ether–ethanol solution, was used to form a support matrix in the lipophilic *n*-octanol membrane. The dry weight of the fully dry collodion solution was determined to be 4% (w/v). Ten millilitres of the collodion solution was diluted with 6 ml of ether–ethanol (vol. ratio 8.5:1.5) solution and 4 ml of *n*-octanol or *n*-dodecanol added to this solution (Neubert and Fürst, 1989). The dry cellophane membrane (4.4 cm × 4.5 cm for membranes with MWCO 12,000–14,000 Da) was fixed with miniature washing clips to a string in a vertical position, and the octanol/nitrocellulose solution was poured three times (approximately 1.5 ml each time) on to the upper ridge of the cellophane membrane at 15 min intervals to form the fused membrane. The fused membrane was left to dry over night. In the initial experiments the octanol/nitrocellulose solution was poured from one up to seven times over the membrane. The weight ratio of *n*-octanol and nitrocellulose, as well as of *n*-dodecanol and nitrocellulose, in the dry lipophilic octanol/nitrocellulose, and dodecanol/nitrocellulose, membrane was approximately 10:1.

The density of nitrocellulose, as well as the amount of nitrocellulose in the collodion solution from Fluka, was estimated by assuming that all ethanol and diethyl ether did evaporate during drying the only residue being solid nitrocellulose. Exact volume of the collodion solution was weighed into a volumetric flask and evaporated to dryness. Then water was added to the flask and the volume of the sample determined from the weight of water added and its density. The amount of nitrocellulose in the collodion solution was determined to be 4.2% (w/v) and its density to be 0.872 ± 0.190 g/ml (mean and S.D. of six experiments). This is not far from the density of *n*-octanol, 0.827 g/ml, or *n*-dodecanol 0.831 g/ml (Merck Index, 2001; Saleh et al., 2004). Since nitrocellulose was only about 10% of the weight of the dry lipophilic membrane, and since the density of nitrocellulose was determined to be quite similar to that of octanol and dodecanol,

the thickness of the lipophilic membranes was determined from the weight of the membrane and the density of either *n*-octanol or *n*-dodecanol.

2.3. Permeation experiments

Drug flow through our membranes was measured in Franz-diffusion cells (FDC400 15 FF) from Vangard International Inc. (Neptune, USA) where the donor phase (2 ml) is unstirred but the receptor phase (12 ml) is stirred with a magnetic stirrer during the experiment. The area of the exposed membrane is 1.77 cm^2. The donor phases consisted of water or aqueous 1–15% (w/v) HPβCD solution containing sodium nitrate or drug in a solution or suspension. The receptor phase contained from 1% to 10% (v/w) HPβCD octanol saturated aqueous solution. In all cases a linear increase in drug concentration was observed in the receptor phase indicating that the barrier function of the membrane was constant during course of the experiment. The solubility of water in octanol is 4% (w/v). The membrane was allowed to equilibrate with both the aqueous receptor phase and small amount of the aqueous donor phase for about 15 min before the donor phase containing the drug was added to the donor side of the membrane. The flux through the membrane was determined at room temperature (22–23 °C). Samples were withdrawn from the receptor phase at various time points up to 4 h and the flux was calculated from each permeability profile. All permeation coefficients were calculated from the flux and the total concentration of dissolved drug (i.e. both free and bound in a dissolved HPβCD complex) in the donor phase. The results presented are the means and the S.D. of from three to over six separate experiments. High performance liquid chromatography (HPLC) was used for quantitative determination of the permeating compounds in the donor and receptor phases except nitrate was determined by a nitrate selective electrode (Másson et al., 2002).

2.4. Chromatographic determinations

The quantitative determination of drugs were performed on HPLC equipment consisting of a Merck-Hitachi AS-2000A autosampler, Merck-Hitachi L-6200A pump and a Merck-Hitachi L-4250 lamp fixed wave length UV detector. The column used was a C18, reversed phase column (Phenomex, USA), 150 mm, 4.6 mm 1D, 5 μm bead. The flow rate was 1.5 ml/min. The mobile phases (volume ratios), wavelengths and retention times were as follows: Hydrocortisone—acetonitrile, tetrahydrofuran and water (33:1:66), 254 nm, 3.4 min. Naproxen—acetonitrile, acetic acid and water (65:1:34), 272 nm, 2.0 min. Salicylic acid—acetonitrile, acetic acid and water (30:1:69), 295 nm, 4.8 min. Lidocaine—methanol, triethylamine and water (70.1:29), 254 nm, 3.9 min. 17β-Estradiol—acetonitrile and water (55:45), 285 nm, 2.4 min. Triclosan—acetonitrile and water (70:20), 280 nm, 4.4 min.

2.5. Quantitative determination of octanol

The quantitative determination of *n*-octanol in cyclodextrin containing aqueous solutions has previously been described (Másson et al., 2005). Briefly toluene was added to aliquots of *n*-octanol saturated aqueous cyclodextrin solutions. These mixtures were thoroughly shaken for 2 h and then centrifuged. The octanol concentration in the organic phase was measured with a Varian 3800 Gas Chromatography equipment (Varian, Palo Alto, USA) using a thermal conductivity detector polarized capillary column, and He gas as a mobile phase. Standard curve was obtained with octanol standards diluted in methanol or hexanol. Hexanol was used as internal standard.

2.6. Determination of partition coefficients

The procedure for determining the observed partition coefficients ($K_{o/w\,obs}$) for drug distribution between *n*-octanol and a cyclodextrin containing aqueous solutions has previously been described (Másson et al., 2005). Briefly a 3 ml aliquot of an octanol solution of the drug was transferred to 10 ml vials containing 3 ml octanol saturated aqueous cyclodextrin solution or aqueous buffer solution. The vials were shaken, with a mechanical shaker, for 5–20 h at room temperature. The phases were then separated by centrifugation and samples from each phase were analyzed be HPLC to determine the drug concentrations. The partition coefficient is the drug concentration ratio between octanol and the aqueous phase at the specified pH and cyclodextrin concentrations.

3. Theoretical background

Since the permeability constants are the reciprocals of the resistance and since serial resistance is additive the following equation is obtained:

$$J = PC_V = (R_D + R_M + R_R)^{-1} C_V = \left(\frac{1}{P_D} + \frac{1}{P_M} + \frac{1}{P_R}\right)^{-1} C_V \quad (1)$$

where J is the flux of the compound through the membrane, P the overall permeability coefficient, C_V the concentration of the compound in the vehicle (i.e. donor phase), R_D, R_M and R_R are the resistances in the unstirred water layer at the donor side, within the membrane and in the unstirred water layer at the receptor side, respectively, and P_D, P_M and P_R are the permeability coefficients in the unstirred water layer at the donor side, within the membrane and in the unstirred water layer at the receptor side, respectively. In this study R_R was assumed to be negligible due to stirring of the receptor phase (i.e. when the smooth octanol membrane was on the receptor side) and then Eq. (2) is obtained from Eq. (1):

$$J = \left(\frac{P_D P_M}{P_D + P_M}\right) C_V \quad (2)$$

If the value of the permeability constant through the lipophilic membrane (P_M) is much greater than the value of the permeability constant through the diffusion layer (P_D) then Eq. (2) becomes:

$$J = \left(\frac{P_D P_M}{P_D + P_M}\right) C_V \sim \left(\frac{P_D P_M}{P_M}\right) C_V = P_D C_V \quad (3)$$

and the unstirred water layer becomes the main barrier, i.e. the permeation is diffusion-controlled. On the other hand, if P_D is much greater than P_M then Eq. (4) is obtained:

$$J = \left(\frac{P_D P_M}{P_D + P_M}\right) C_V \sim \left(\frac{P_D P_M}{P_D}\right) C_V = P_M C_V \tag{4}$$

and the permeation will be membrane controlled. Finally the diffusion coefficient (D) can be estimated from the Stokes–Einstein equation:

$$D = \frac{RT}{6\pi\eta r N} \tag{5}$$

and

$$P = \frac{DK}{h} \tag{6}$$

where R is the molar gas constant, T the absolute temperature, η the apparent viscosity within the unstirred water layer or the lipophilic membrane, r the radius of the permeating drug molecule, N the Avogadro's number, K the partition coefficient and h is the thickness of the unstirred water layer or the membrane. Thus, the diffusion constant within the unstirred water layer (D_D) will decrease with increasing viscosity of the layer as well as with increasing molecular weight of the drug. For example, small lipophilic drug molecules frequently possess a large permeability coefficient through the lipophilic membrane (i.e. large P_M value) and, thus, may be able to permeate across the lipophilic membrane much faster that they can be transported through the unstirred water layer. Under such conditions diffusion through the water layer becomes the rate-limiting step in the absorption process.

Diffusion coefficients of small molecules in pure water (D_w) at room temperature can be calculated by the empirical formula (Avdeef et al., 2004):

$$\log D_w = -4.113 - 0.4609 \log \text{MW} \tag{7}$$

where MW is the molecular weight of the diffusing molecule. However, the value of D_w in bulk of the aqueous solution will not be identical to the one in the unstirred aqueous layer. Many biological membranes have an aqueous mucus layer or other aqueous layers (e.g. tear film or saliva) adjacent to the membrane surface. These aqueous layers can contain proteins and polysaccharides (e.g. mucins) that bind water molecules into gel-like structures of relatively high η and, since P decreased with increasing η (Eqs. (5) and (6)), these layers are more effective diffusion barriers (i.e. lower P) than pure unstirred water layers. Hydrophilic membrane surfaces will also lead to formation of structured water layers, of relatively high viscosity, at the membrane surface.

4. Results and discussion

4.1. Preparation of the membrane

The fused membranes are relatively simple to prepare and the preparation method gave consistent and reproducible results. During the initial preparation of the membranes the effect of the octanol/nitrocellulose ratio and the thickness of the membrane on the drug flux through the membrane were investigated. No hydrocortisone permeation was observed when the octanol/nitrocellulose ratio was 1:1, as described with dodecanol (Neubert and Fürst, 1989). Increasing the octanol/nitrocellulose ratio to 2:1 resulted in a steady hydrocortisone flow through the membrane. Increasing the octanol/nitrocellulose ratio further to 5:1, 10:1, 20:1 or 40:1 did not result in further increase in the hydrocortisone flux through the membrane. Thus it was decided to keep the octanol/nitrocellulose weight ratio at 10:1 in the dry lipophilic membrane. The effect of the membrane thickness on the permeability coefficient was tested by pouring the octanol/nitrocellulose solution up to seven times over the membrane (Table 1). Hydrocortisone, with MW of 362.5 Da, $\log K_{o/w}$ of 1.6 (between pure water and n-octanol) and $\log D_w$ of -5.29 (estimated from Eq. (7)), permeates the fused membrane predominately via diffusion-controlled mechanism (Eq. (3)) and thus, increasing the thickness of the lipophilic octanol membrane does not have any detectable effect on the permeability constant through the membrane. The permeability constant remains constant at about 4×10^{-6} cm s^{-1} and it was unaffected by the thickness of the lipophilic octanol/nitrocellulose membrane from 0 to 270 μm (Table 1). The ionized form of naproxen permeates the membrane via membrane controlled mechanism (Eq. (4)) and, thus, its permeation is strongly affected by the thickness of the lipophilic octanol/nitrocellulose membrane (Table 1). As do nitrite ions that readily permeate the semi-permeable cellophane membrane ($P = (83.4 \pm 4.0) \times 10^{-6}$ cm s^{-1})

Table 1
The effect of number of octanol/nitrocellulose layers on the permeability coefficient (P) of hydrocortisone and ionized naproxen through the membrane

Number of layers	Lipophilic membrane thickness (±S.D.) (μm)	Permeability coefficient ± S.D. ($\times 10^6$ cm s^{-1})	
		Hydrocortisone	Naproxen
0	0	4.12 ± 0.42	3.10 ± 0.61
1	40 ± 8	3.69 ± 1.33	1.08 ± 0.23
3	120 ± 20	4.69 ± 0.71	0.52 ± 0.09
5	230 ± 50	4.03 ± 0.38	<0.2
7	270 ± 20	3.74 ± 0.21	<0.1

Mean and S.D. from at least six experiments. The donor phase consisted of aqueous 5% (w/v) HPβCD hydrocortisone saturated solution (hydrocortisone concentration, 9.8 mg/ml; pH 6) or solution of naproxen (2.8 mg/ml) and 5% (w/v) HPβCD in aqueous sodium hydroxide solution (pH 10). The receptor phase was an octanol saturated aqueous 10% (w/v) HPβCD solution. Semi-permeable cellophane membrane used had molecular weight cutoff of 12,000–14,000, fused with a 0–270 μm thick octanol/nitrocellulose membrane on the receptor side.

Table 2
Permeation of hydrocortisone ($K_{o/w}$ = 40; MW 362 Da) through an artificial membrane consisting of semi-permeable cellophane membrane, semi-permeable cellophane membrane with a fused octanol/nitrocellulose membrane at the receptor side, and a semi-permeable cellophane membrane with a fused dodecanol/nitrocellulose membrane

Property	Cellophane	Cellophane/octanol	Cellophane/dodecanol
Thickness (±S.D.) (μm)			
Diffusion layer	230 ± 10	210 ± 10	∼230
Lipid membrane	0	120 ± 20	140 ± 10
Permeability coefficient ($\times 10^{-6}$ cm s^{-1}) (mean ± S.D.)	4.12 ± 0.42	4.69 ± 0.71	1.17 ± 0.33

Mean and S.D. of at least six experiments. The donor phase consisted of an aqueous 5% (w/v) HPβCD solution saturated with hydrocortisone (hydrocortisone concentration, 9.8 mg/ml; pH 6) and the receptor phase consisted of an octanol saturated or dodecanol saturated aqueous 10% (w/v) HPβCD solution. The MWCO of cellophane membranes was 12,000–14,000 Da.

but their permeation is almost blocked completely when octanol/nitrocellulose membrane (120 ± 20 μm) is fused on the receptor side ($P = (0.02 \pm 0.02) \times 10^{-6}$ cm s^{-1}). Pouring the octanol/nitrocellulose solution three times over the semi-permeable cellophane membrane resulted in about 120 μm thick octanol membrane (Tables 1 and 2), which is the thickness of the octanol/nitrocellulose membrane in the following experiments. During repeated preparation of the membrane during a 1-year period the thickness of the membrane remained fairly constant at 120 μm with a S.D. of only 20 μm. The thickness of the hydrated cellophane membrane was determined to be about 210–230 μm and the total thickness of the cellophane membrane with the fused octanol/nitrocellulose membrane was about 350 μm.

Hydrocortisone permeates the octanol/nitrocellulose membrane more readily than the dodecanol/nitrocellulose membrane (Table 2). The solubility of hydrocortisone is comparable in the two lipophilic membranes, about 4 mg/ml in *n*-octanol (Ruelle et al., 2000) and 4.6 mg/ml in *n*-dodecanol (Bendas et al., 2003), but the physicochemical properties of the two solvents used to prepare the membranes are different, especially their melting point, which can explain the differences in permeability (Table 2). The melting point of *n*-octanol is −17 to −16 °C and its viscosity at 30 °C is 3.45 cP while the melting point of *n*-dodecanol is 24 °C and its viscosity at 30 °C is 6.51 cP (Merck Index, 2001; Saleh et al., 2004; Oswal et al., 2005). Thus, under the experimental conditions (22–23 °C) dodecanol was in a solid or very viscous semisolid state and, according to Eq. (5), the higher viscosity of dodecanol results in smaller hydrocortisone diffusion coefficient through the lipophilic membrane. The relative high viscosity of the dodecanol/collodion solution at room temperature also made preparation of a homogeneous dodecanol membrane somewhat more difficult compared to preparation of the octanol membrane.

When hydrocortisone permeation through different semi-permeable cellophane membranes was compared, the fastest permeation was obtained through a membrane with a MWCO 12,000–14,000 Da but the lowest through a membrane with a MWCO of 3500 Da (Table 3). Due to low viscosity of the lipophilic octanol/nitrocellulose membrane the permeation of lipophilic drug molecules, through the lipophilic part of the fused membrane will be relatively fast. Thus it is preferable that the flux through the hydrated part of the fused membrane should also be fast. The most permeable MWCO 12,000–14,000 Da cellophane membrane was therefore selected for further investigation.

4.2. The effect of donor phase composition

The cellophane membrane formed the unstirred water layer of the fused membrane that, in case of the hydrocortisone permeation, formed the rate-limiting barrier. Thus, fusion of a lipophilic octanol/nitrocellulose membrane on the receptor side of the membrane had no or insignificant effect on hydrocortisone permeation through the membrane. Reversing the membrane (i.e. having the lipophilic membrane on the donor side) did not affect the permeation when the HPβCD concentration was identical in both the donor and the receptor phase (5% HPβCD). In this case the permeability coefficient through the simple cellophane membrane was not significantly different from the permeability coefficient through the octanol/nitrocellulose membrane (Table 3). However, when HPβCD concentration

Table 3
The effect of the molecular weight cutoff (MWCO) of the cellophane membrane, concentration of HPβCD in the receptor phase and the position of the octanol/nitrocellulose membrane on the permeability coefficient (P) of hydrocortisone through the membrane

HPβCD concentration (%, w/v) in receptor phase	MWCO (Da) cellophane membrane	P ± S.D. ($\times 10^{6}$ cm s^{-1})		
		Only cellophane	Octanol membrane below	Octanol membrane above
10	3500	1.43 ± 0.13	1.29 ± 0.03	2.00 ± 0.16
10	6000–8000	2.88 ± 0.37	3.04 ± 0.08	4.01 ± 0.15
10	12000–14000	4.12 ± 0.42	4.69 ± 0.71	8.34 ± 0.41
5	12000–14000	5.32 ± 0.45	5.02 ± 0.04	6.25 ± 0.77
1	12000–14000	5.18 ± 0.20	4.95 ± 0.20	4.55 ± 0.39

Mean and S.D. of at least three independent experiments. The donor phase consisted of aqueous 5% (w/v) HPβCD solution saturated with hydrocortisone (i.e. 9.8 mg hydrocortisone/ml donor phase, pH 6). The receptor phase consisted of octanol saturated aqueous 1–10% (w/v) HPβCD solution.

Table 4
The effect of HPβCD concentration in the receptor and donor phase on hydrocortisone flux and permeability coefficient (*P*) through the semi-permeable cellophane membrane and through the cellophane with an octanol/nitrocellulose membrane fused on the receptor side

HPβCD concentration (%, w/v)		Flux ± S.D. ($\mu g\,h^{-1}\,cm^{-2}$)		P ± S.D. ($\times 10^6\,cm\,s^{-1}$)	
Donor	Receptor	Cellophane	Cellophane/octanol	Cellophane	Cellophane/octanol
10	10	203.9 ± 8.0	183.2 ± 12.5	3.66 ± 0.30	3.44 ± 0.23
5	10	147.5 ± 12.0	162.4 ± 20.2	4.12 ± 0.42	4.69 ± 0.71
5	5	192.6 ± 14.1	172.4 ± 1.3	5.41 ± 0.44	5.02 ± 0.04
1	10	46.63 ± 2.70	71.75 ± 3.40	6.68 ± 0.75	11.4 ± 0.50
1	1	74.20 ± 6.50	71.11 ± 7.70	9.82 ± 1.19	9.15 ± 0.99

The results presented are the mean values and S.D. of at least three experiments. The cellophane membrane had MWCO of 12,000–14,000 Da. The donor phase was saturated with hydrocortisone and the permeability coefficient was calculated from the determined hydrocortisone solubility in the donor phase.

in the receptor phase was 10% the hydrocortisone permeation through the reverse membrane was faster than trough the simple cellophane membrane or an octanol/nitrocellulose membrane with the lipophilic membrane on the receptor side. 2-Hydroxypropyl-β-cyclodextrin (MW about 1400 Da) can easily permeate the MWCO 12,000–14,000 Da semi-permeable cellophane membrane. When HPβCD concentration in the receptor phase is higher than in the donor phase HPβCD molecules will flow from the receptor phase through the cellophane membrane and into the donor phase and, thus, form a concentration gradient within the membrane. However, HPβCD is not able to permeate the lipophilic octanol/nitrocellulose membrane. Therefore the HPβCD concentration within the cellophane section of the fused membrane will be in equilibrium with the concentration in either the donor phase or the receptor phase, depending on which phase is in direct contact with the cellophane membrane. When the fused membrane is placed in the diffusion cell with lipophilic membrane on the donor side then the partition coefficient ($K_{o/w\,obs}$) for partitioning of the drug molecules into the cellophane membrane will be dependent on the HPβCD concentration in the receptor phase. The drug permeation will therefore increase with increasing HPβCD concentration in receptor phase, whereas changes in HPβCD concentration in receptor phase will have little or no effect when lipophilic membrane is on the receptor side (Table 3).

Cyclodextrin present in the cellophane membrane does increase somewhat the permeation resistance within the membrane (Table 4). Thus the hydrocortisone permeability coefficient through the semi-permeable cellophane membrane is $9.82 \times 10^{-6}\,cm\,s^{-1}$ when HPβCD concentration is 1% (w/v) in both the donor and the receptor phase but only $3.66 \times 10^{-6}\,cm\,s^{-1}$ when the HPβCD concentration is increased to 10% (w/v). Nonetheless, the hydrocortisone flux is increased when the saturation concentration of hydrocortisone in the donor phase is increased by increasing the HPβCD concentration (Table 4 and Fig. 1). Since HPβCD permeates through the simple cellophane membrane the drug permeability coefficient can be affected by the HPβCD concentration in both the donor and the receptor phase. The permeation through the cellophane membrane with a fused octanol/nitrocellulose membrane on the receptor side is only determined by the cyclodextrin concentration in the donor phase. Under such conditions the cyclodextrin concentration in the receptor phase has no effect as long as it is sufficient to maintain sink conditions (Table 4). The solubility of *n*-octanol in 1%, 5% and 10% (w/v) aqueous HPβCD solution was determined to be 0.2, 0.9 and 1.5 mg/ml, respectively. The receptor phase was saturated with octanol and, thus, did not extract octanol from the octanol membrane. The donor phase was not in direct contact with the octanol membrane, except when the membrane was in a reverse position with the octanol membrane on the donor side. Under such conditions octanol extracted from the membrane could reduce hydrocortisone complexation and that could partly explain the somewhat larger permeability through the reversed membranes with the fused octanol/nitrocellulose membrane on the donor side (Table 3). Maximum amount of octanol that a 2 ml of aqueous 10% (w/v) HPβCD donor phase could extract from the membrane is 3 mg or about 2% of the octanol membrane. It is unlikely that this small amount could have effect on drug permeability through the membrane.

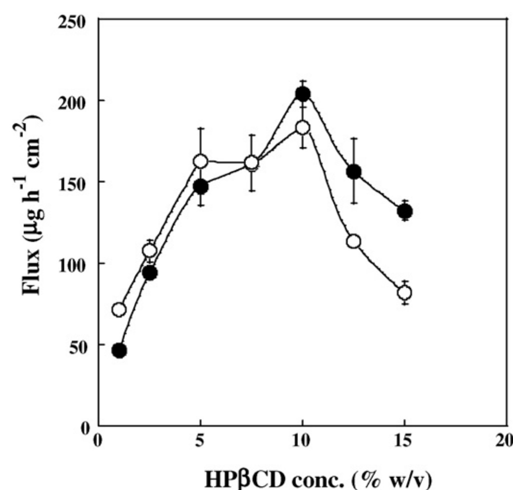

Fig. 1. The effect of HPβCD concentration on the flux of hydrocortisone from an aqueous HPβCD solution containing 16 mg/ml of hydrocortisone trough a semi-permeable cellophane membrane (MWCO 12,000–14,000 Da) with (○) or without (●) fused octanol/nitrocellulose membrane. At HPβCD concentrations below 10% the drug was in suspension in the donor phase but in solution at HPβCD concentrations of 10% and higher. The receptor phase was in all cases an aqueous 10% (w/v) HPβCD solution saturated with octanol. Mean values and S.D. of at least three experiments.

4.3. The permeation pattern

Fig. 1 shows the effect of HPβCD concentration, and the degree of drug saturation in the donor phase, on the flux of hydrocortisone through both semi-permeable cellophane membrane and the same cellophane membrane with a fused octanol/nitrocellulose membrane on the receptor side. The donor phase contained constant amount of hydrocortisone (16 mg/ml) in a suspension at HPβCD concentrations below 10% (w/v) but in solution at HPβCD concentration of 10% or higher. At HPβCD concentrations below 10%, when the donor phase is saturated with hydrocortisone, the flow of drug molecules through the membrane is diffusion-controlled and the flux through both types of membranes increases with increasing HPβCD concentration. When the HPβCD concentration in the donor phase is equal or higher than the saturation concentration (i.e. when the HPβCD concentration, in the donor phase is >10%) the permeation through the fused membrane becomes membrane controlled and, consequently, the flux through the membrane is smaller than through the semi-permeable cellophane membrane. Identical permeation patterns have been observed when permeation of lipophilic drugs from aqueous cyclodextrin solutions through various biological membranes were investigated (Loftsson et al., 2003, 2006). It should also be mentioned that cyclodextrins have been shown to form aggregates at high cyclodextrin concentrations and that the aggregate formation can decrease drug permeability through semi-permeable cellophane membranes (Loftsson et al., 2002, 2004).

Table 5
Permeation of various drugs through semi-permeable cellophane or semi-permeable cellophane membrane with a fused octanol/nitrocellulose membrane

Membrane	Donor phase	pH[a]	$f_{unionized}$[b]	$\log K_{o/w\,obs}$[c]	$P \pm$ S.D. ($\times 10^6$ cm s^{-1})[d]
Drug[e]					
Sodium nitrate (MW 85.0; C_V = 10 mg/ml)					
Cellophane	Pure water	7	0.00	–	83.4 ± 4.0
Cellophane/octanol	Pure water	7	0.00	–	0.02 ± 0.02
Hydrocortisone (MW 362.5; $\log D_w$ −5.29; C_V = 9.8 mg/ml)					
Cellophane	5% HPβCD	6	1.00	0.4	4.12 ± 0.42
Cellophane/octanol	5% HPβCD	6	1.00	0.4	4.69 ± 0.71
Naproxen (MW 230.3; pK_a 4.2; $\log D_w$ −5.20; C_V = 2.8 mg/ml)					
Cellophane	5% HPβCD, HCl	2.0	0.99	1.9	4.37 ± 0.30
Cellophane/octanol	5% HPβCD, HCl	2.0	0.99	1.9	3.75 ± 0.32
Cellophane	5% HPβCD, NaOH	10	0.00	−1.5	3.60 ± 0.21
Cellophane/octanol	5% HPβCD, NaOH	10	0.00	−1.5	0.52 ± 0.09
Salicylic acid (MW 138.1; pK_a 2.8; $\log D_w$ −5.10; C_V = 1.8 mg/ml)					
Cellophane	Dilute HCl	1.5	0.96	2.2	45.7 ± 1.3
Cellophane/octanol	Dilute HCl	1.5	0.96	2.2	21.6 ± 1.7
Cellophane	Acetate buffer	4.0	0.06	1.0	41.1 ± 0.8
Cellophane/octanol	Acetate buffer	4.0	0.06	1.0	16.7 ± 1.9
Cellophane	Dilute NaOH	7.5	0.00	−0.4	33.7 ± 0.3
Cellophane/octanol	Dilute NaOH	7.5	0.00	−0.4	0.28 ± 0.05
Lidocaine (MW 234.3; pK_a 7.9; $\log D_w$ −5.21; C_V = 1.8 mg/ml)					
Cellophane	Acetate buffer	4.0	0.00	−1.0	24.8 ± 1.9
Cellophane/octanol	Acetate buffer	4.0	0.00	−1.0	2.12 ± 0.26
Cellophane	Dilute NaOH	7.5	0.29	2.0	26.4 ± 0.3
Cellophane/octanol	Dilute NaOH	7.5	0.29	2.0	3.35 ± 0.66
Cellophane	Dilute NaOH	8.5	0.80	2.2	26.1 ± 0.9
Cellophane/octanol	Dilute NaOH	8.5	0.80	2.2	5.77 ± 0.32
17β-Estradiol (MW 272.4; $\log D_w$ −5.24; C_V = 2.8 mg/ml)					
Cellophane	5% HPβCD	7	1.0	1.5	3.86 ± 0.27
Cellophane/octanol	5% HPβCD	7	1.0	1.5	3.82 ± 0.41
Triclosan (MW 289.5; pK_a 7.9; $\log D_w$ −5.25; C_V = 2.0 mg/ml)					
Cellophane	5% HPβCD	5	1.00	3.8	3.64 ± 0.36
Cellophane/octanol	5% HPβCD	5	1.00	3.8	1.75 ± 1.04
Cellophane	5% HPβCD, NaOH	10	0.01	2.3	2.71 ± 0.48
Cellophane/octanol	5% HPβCD, NaOH	10	0.01	2.3	1.22 ± 0.50

The cellophane membranes had a 12,000–14,000 Da MWCO. Mean and S.D. is from at least three experiments.

[a] pH is the determined pH of the donor phase.

[b] $f_{unionized}$ is the fraction of unionized species in pure water at the given pH.

[c] $\log K_{o/w\,obs}$ is the logarithm of determined partition coefficient between *n*-octanol and the donor phase at given pH and HPβCD concentration.

[d] P is the permeability coefficient (mean ± S.D. of at least three determinations).

[e] Logarithm of the diffusion coefficient ($\log D_w$) of the unionized form calculated according to Eq. (7). C_V is concentration of the permeating compound in the donor phase (vehicle).

4.4. The effect of lipophilicity

Table 5 shows the permeability of nitrate and various drugs through both the semi-permeable cellophane membrane with MWCO 12,000–14,000 Da and the same semi-permeable cellophane membrane with a fused octanol/nitrocellulose membrane on the receptor side. All compounds permeate the cellophane membrane and their permeability coefficient appears to be proportional to their calculated diffusion coefficient ($\log D_{\mathrm{w}}$) in pure water. Since permeation through the cellophane membrane is diffusion-controlled ionization of the permeating molecule has relatively little effect on their permeability coefficient. In contrast permeation of hydrophilic molecules through the semi-permeable cellophane membrane with a fused octanol/nitrocellulose membrane on the receptor side is membrane controlled. Hence, the very hydrophilic nitrate ion permeates several thousand times faster through the cellophane membrane than through the octanol membrane. Permeation of larger ionizable molecules, where the ionized species are couple of hundred to couple of thousand times more hydrophilic than the unionized form ($\Delta\log K_{\mathrm{o/w}} = 2$–$4$), is very sensitive towards pH of the donor phase. Permeation of the unionized form is mainly diffusion-controlled, whereas permeation of the ionized form is mainly lipophilic membrane controlled (Table 5). However, the permeation coefficient of protonized lidocaine is higher than expected based on the lipophilicity of the molecule and the permeability coefficient of the unionized drug. Protonized lidocaine is the only cationic drug tested and it does possess some surface activity, both of which could influence its partition into the membrane. Also, the pH given in Table 5 is the pH in the bulk solution that could be different from the pH in the aqueous diffusion layer immediate to the octanol membrane. Fig. 2 shows the relationship between the permeation coefficient (P) and the octanol/vehicle partition coefficient ($K_{\mathrm{o/w\,obs}}$). It shows that the permeation coefficient through the semi-permeable cellophane membrane is practically independent of the lipophilicity of the naproxen molecule, whereas the permeation through the same cellophane membrane with the fused octanol/nitrocellulose membrane is strongly influenced by the lipophilicity of the molecule. Both the ionized and the unionized form of triclosan are relatively lipophilic, the unionized form is about 30-times more lipophilic than the ionized form as determined by $K_{\mathrm{o/w\,obs}}$ between the vehicle (i.e. the donor phase) and n-octanol (Table 5), and both forms permeate the lipophilic membrane relatively well although the permeability coefficient of the ionized form is somewhat lower than that of the unionized form.

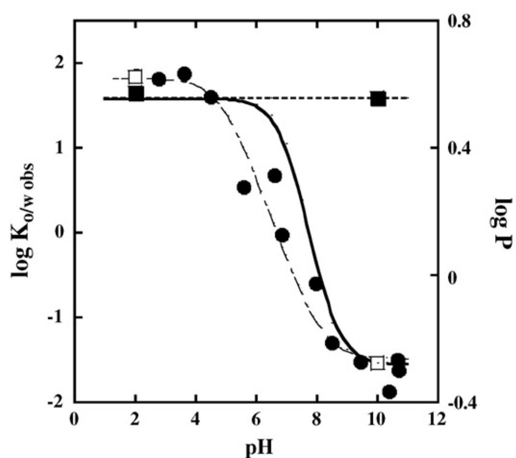

Fig. 2. Logarithm of the experimentally determined permeability coefficients ($\log P$) for naproxen (pK_{a} 4.2) through semi-permeable cellophane membrane (MWCO 12,000–14,000 Da) with (□) or without (■) fused octanol/nitrocellulose membrane, and the experimentally determined partition coefficient between n-octanol and aqueous 5% (w/v) HPβCD solution (●) ($\log K_{\mathrm{o/w\,obs}}$) at room temperature. The donor phase in the permeation studies consisted of aqueous 5% (w/v) HPβCD solution containing 2.7 mg/ml naproxen.

5. Conclusion

Drug molecules encounter two types of resistance when they permeate through biological membranes, i.e. membrane resistance in the lipophilic membrane and diffusion resistance in the unstirred water layers adjacent to both surfaces of the lipophilic membrane. There is a need for a simple artificial membrane that mimics these physiochemical properties of biological membranes. Simple membranes that can be used during development of pharmaceutical formulation to test the effects of various excipients on drug availability from, for example, eye drops, nasal sprays, creams and ointments. We have developed an artificial membrane that is cheap and simple to prepare. The thickness and composition of the unstirred water layer is standardized through usage of hydrated semi-permeable cellophane membrane. The lipophilic part of the membrane consists of n-octanol (90%) in a nitrocellulose matrix (10%) without addition of surfactants or other additives. The lipophilic membrane is protected from the formulation by the hydrated cellophane membrane on the donor side. The membrane can be used in currently used diffusion cells such as the Franz diffusion cell. The fused membrane also offers the advantage that the two layers can be studied separately. The permeability coefficient for the hydrated membrane can readily be determined and the partition coefficient for the lipophilic membrane should be almost identical to the experimentally determined $K_{\mathrm{o/w}}$.

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